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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 31/535, C07D 265/38 | A1

(11) International Publication Number: WO 93/03729

(43) International Publication Date: 4 March 1993 (04.03.93)

(21) International Application Number: PCT/US92/06681

(22) International Filing Date: 10 August 1992 (10.08.92)

(30) Priority data:
744,619 12 August 1991 (12.08.91) US

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(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE).

Published
With international search report.

(54) Title: N-SUBSTITUTED PHENOXAZINES FOR TREATING MULTIDRUG RESISTANT CANCER CELLS

(57) Abstract

Phenoxazines, unsubstituted or N-substituted as defined herein, can potentiate the antitumor effectiveness of chemotherapeutic agents, particularly in multiple drug resistant (MDR) cells.

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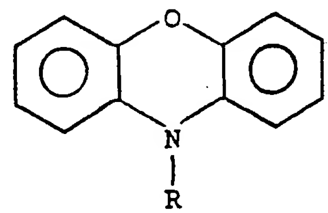
N-SUBSTITUTED PHENOXAZINES FOR TREATING MULTIDRUG RESISTANT CANCER CELLS

The present invention is directed to chemotherapy of cancer.

A major reason for failure of treatment of cancer patients is resistance to conventional chemotherapeutic agents. One type of drug resistance, called multi-drug resistance (MDR) is characterized by cross-resistance to functionally and structurally unrelated chemotherapy drugs, such as doxorubicin, vincristine (VCR), vinblastine (VLB), colchicine, and actinomycin D. A number of drugs appear to be active in modifying MDR in model systems, including the calcium channel blocker, verapamil (VRP), the calmodulin inhibitor, trifluoperazine, the anti-arrhythmic drug, quinidine, reserpine, cyclosporin A, <u>Vinca</u> alkaloid analogs, dihydropyridines, and pyridine analogs. Thus, it can be seen that agents that reverse MDR apparently do not seem to have common features. Although several of these MDR-reversing agents have been or are now being tested clinically in cancer patients, they have largely failed to enhance sensitivity to the chemotherapeutic agent. Instead, serious toxicities develop at or below plasma drug levels required for MDR reversal in vitro.

A tricyclic compound, phenoxazine, has been found to potentiate the uptake of VCR and VLB in MDR GC_3/Cl and $KBCh^R$-8-5 cells to a greater extent than verapamil. While this discovery has utility and holds promise, it would be desirable to identify derivatives of phenoxazine which would modulate MDR and which show even higher stability and lower toxicity.

In one aspect, the present invention comprises compounds of formula (1):

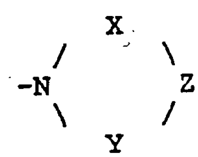


(1)

and pharmacologically acceptable salts thereof, wherein R is $-[C(O)]_a-(CH_2)_b-A$; wherein a is 0 or 1 and b is an integer from 0 to 6, provided that a and b are not both zero;

A is selected from the group consisting of

$-NR_1R_2$ wherein R_1 and R_2 are independently alkyl having 1 to 4 carbon atoms, and either or both of R_1 and R_2 are optionally substituted with -OH;

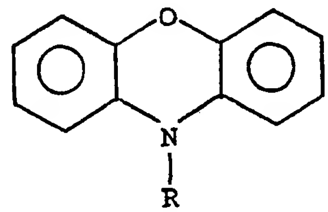


wherein X and Y are independently alkylene having 1 to 4 carbon atoms, and Z is -O-, $-N(R_3)$-or $-CH(R_4)-$, wherein R_3 is hydrogen or alkyl having 1 to 4 carbon atoms optionally substituted with a hydroxyl group, and wherein R_4 is hydrogen or alkyl having 1 to 4 carbon atoms optionally substituted with a hydroxyl groups;

halide; and trihalomethyl.

The present invention also relates to a method of potentiating the cytotoxicity of an agent cytotoxic to a tumor cell, comprising administering to said tumor cell, while it is exposed to said cytotoxic agent, a potentiating agent in an amount effective to potentiate the cytotoxicity of said cytotoxic agent to said cell,

wherein said potentiating agent comprises a compound of formula (1):



(1)

or a pharmacologically acceptable salt thereof, wherein R is -H or $-[C(O)]_a-(CH_2)_b-A$; wherein a is 0 or 1 and b is an integer from 0 to 6, provided that a and b are not both zero; and

A is selected from the group consisting of

$-NR_1R_2$ wherein R_1 and R_2 are independently alkyl having 1 to 4 carbon atoms, and either or both of R_1 and R_2 are optionally substituted with -OH;

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      X
    /   \
 -N       Z
    \   /
      Y
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wherein X and Y are independently alkylene having 1 to 4 carbon atoms, and Z is -O-, $-N(R_3)-$or $-CH(R_4)-$, wherein R_3 is hydrogen or alkyl having 1 to 4 carbon atoms optionally substituted with a hydroxyl group, and wherein R_4 is hydrogen or alkyl having 1 to 4 carbon atoms optionally substituted with a hydroxyl group;

halide; and trihalomethyl.

The present invention further relates to a composition comprising cytotoxic agent toxic to tumor cells, and a potentiating agent which potentiates the cytotoxicity of said cytotoxic agent, wherein said potentiating agent comprises a compound of formula (1) and wherein said cytotoxic agent and potentiating agent

are present in amounts effective to render the composition cytotoxic to tumor cells.

The present invention still further relates to a method of killing a tumor cell which comprises administering to said cell a composition as described above in an amount effective to kill said cell.

As described in more detail below, the present invention provides novel and effective means for potentiating the desired cytotoxic effect of anticancer drugs in tumor cells and especially in multidrug-resistant (MDR) cells.

One preferred group of compounds of the formula (1) is the N-alkyl derivatives, in which a is 0 in formula (1). Of those compounds wherein a is 0, the more preferred include those in which b is 3 or 4, denoting unbranched propylene and butylene moieties; R_1 and R_2 each are ethyl, n-propyl, ω-hydroxyethyl, or ω-hydroxypropyl; X and Y are each $-CH_2-$ or $-CH_2CH_2-$ and, more preferably, both X and Y are $-CH_2CH_2-$; and R_3 and R_4 are each -H or ethyl, propyl, e.g. n-propyl, ω-hydroxyethyl or ω-hydroxypropyl. Other more preferred embodiments when a is 0 are those derivatives wherein b is 3 or 4 and A is halogen, preferably chloro.

Another preferred group of compounds of formula (1) is the N-acyl derivatives, in which a is 1 in formula (1). Of those compounds wherein a is 1, the more preferred include those in which b is 1 or 2, more preferably 1; R_1 and R_2 are each ethyl, n-propyl, ω-hydroxyethyl or ω-hydroxypropyl; X and Y are each $-CH_2-$ or $-CH_2CH_2-$, and more preferably, both X and Y are $-CH_2CH_2-$; each of R_3 and R_4 is -H or ethyl, n-propyl, ω-hydroxyethyl or ω-hydroxypropyl. Other more preferred

embodiments are those in which b is 0 or 1 and A is trihalomethyl, preferably trichloromethyl or trifluoromethyl; and in which b is 1 or 2 and A is halogen, preferably chloro.

As used herein, unless specified otherwise, "alkyl" means saturated, branched or unbranched groups of the formula $-(C_nH_{2n+1})$; "halo" or "halogen" means fluoro, chloro, bromo, and/or iodo; and the optional hydroxyl and halo substituents disclosed herein can be on any carbon of an alkyl or alkylene group.

The compounds of this invention form salts, which are also within the scope of the invention, with various inorganic and organic acids. The pharmacologically acceptable acid addition salts of the compounds of the present invention may be prepared by conventional means, such as by reacting with an appropriate acid providing the desired anion, either in a solvent or medium in which the salt is insoluble, or in water. The salts of strong acids are preferred. As exemplary, but not limiting, of pharmacologically acceptable acid salts are the salts of hydrochloric, hydrobromic, sulfuric, nitric, acetic, fumaric, malic, maleic, tartaric and citric acids.

In general, the synthesis of the N-alkyl and N-acyl derivatives is straightforward. N-alkylation can be achieved in the presence of basic condensing agents like sodium amide. The general procedure for preparing the N-alkyl derivatives of formula (1) consists of the condensation of phenoxazine with the appropriate α, ω-di-alkylhalide in such as $Cl-(CH_2)_b-Br$ wherein b is 1 to 6, in the presence of sodium amide, either in liquid ammonia or in an anhydrous solvent such as toluene or

benzene. For instance, the reaction of phenoxazine with mixed chlorobromoalkanes in the presence of sodium amide gives reactive N-chloroalkylphenoxazines, which can then be converted to the desired compound by reaction with an intermediate of the formula H-$(CH_2)_b$-A wherein b and A have the meanings set forth above.

More specifically, compounds such as those described in Examples 1-14 below can be prepared by first alkylating phenoxazine with 1-bromo-3-chloropropane or 1-bromo-4-chloropropane to produce 10-(3'-chloropropyl) phenoxazine or 10-(4'-chlorobutyl)phenoxazine, alkylation being accomplished by first converting phenoxazine to the anionic species using the strong base, sodium amide. Iodide-catalyzed nucleophilic substitution of the propyl or butyl chloride with various secondary amines (e.g. N,N-diethylamine, N,N-diethanolamine, morpholine, piperidine, pyrrolidine and ß-hydroxyethyl-piperazine) by refluxing for about 20 hours with potassium carbonate in anhydrous acetonitrile affords the free bases of formula (1).

The acyl derivatives of formula (1) can be synthesized by acylating phenoxazine with a compound of the formula Cl-C(O)-$C(CH_2)_{0-6}$-Cl and then reacting the product with an amine of the formula H-A, wherein A has the meaning given above in anhydrous acetonitrile containing potassium iodide. The haloacetylphenoxazine can be prepared by reacting phenoxazine with the anhydride $(C(halo)_3CO)_2O$.

All the compounds described in Examples 1-14 were separated and purified by column chromatography or recrystallization and dried under high vacuum. The

structures were established by UV-, IR, ^{1}H- and ^{13}C-NMR and EIMS spectral data, and by elemental analyses. The physical properties of the compounds are given in Table I. The UV-spectral data of N-substituted phenoxazines are in close agreement with the spectral characteristics of analogous heterocycles. The IR bands also indicate the presence of characteristic functional groups, and peaks at 1670-1695 cm^{-1} indicated the presence of >C=O group in the acyl derivatives. The ^{1}H-NMR in $CDCl_3$, typical of phenoxazine compound, showed eight aromatic protons and the data are in accordance with the structures assigned. The assignment of protons is fully supported by the integration curves. The ^{13}C-NMR spectrum of each N-substituted phenoxazine exhibited size signals representing 12 aromatic carbons. The GC-Mass spectrum showed an intense molecular ion peak (M^+) for each of the compounds characteristic of the phenoxazine type of structure. The spectral data are consistent with the assigned structures.

SYNTHESIS AND ANALYSIS

In the syntheses and experiments described below, melting points were recorded on a Perkin-Elmer Model 1320 spectrophotometer, as KBr pellets; UV-spectra were recorded in MeOH on a Perkin-Elmer Lambda 3B spectrophotometer. Elemental analyses were performed and found values within 0.4% of theoretical, unless otherwise noted. Reactions were monitored by tlc. For tlc, Analtech silica gel GF plates (20 x 20 cm, 250 microns, glass-backed), with petroleum ether-ethylacetate (9.7:0.3 by volume, system A), and ethylacetate-methanol (9.9:0.1 by volume, system B) as solvents were used. Column chromatography utilized

silica gel Merc grade 60 (230-400 mesh, 60Å). ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ solution in a 5-mm tube on an IBM NR 200 AF Fourier transform spectrometer with tetramethylsilane as internal standard. Chemical shifts are expressed as "δ" (ppm) values. The spectrometer was internally locked to the deuterium frequency of the solvent. Electron-impact mass spectra (EIMS) were recorded on a Ribermag R10-10C GC-mass spectrometer with an upper mass limit of 1500 AMU. All chemicals and supplies were obtained from standard commercial sources unless otherwise indicated. Phenoxazine, secondary amines indicated in the text, and anhydrous organic solvents were purchased from Aldrich Chemical Co. (Milwaukee, WI). Vincristine sulfate (oncovin) was purchased from Eli Lilly and Co. (Indianapolis, IN), and vinblastine sulfate was from Cetus Corporation (Emeryville, CA). [G-^{3}H]vincristine (sp. act. (specific activity) 7.1 Ci/mmol), and [G-^{3}H]vinblastine (sp. act. 10.1 Ci/mmol) were obtained from Amersham Corporation (Arlington Heights, IL). Verapamil hydrochloride, colchicine, RPMI-1640 medium, powder with glutamine and without sodium bicarbonate were purchased from the Sigma Chemical Co. (St. Louis, MO).

The synthesis of representative compounds of formula (1) is described below. Each of the indicated compounds in these Examples is considered a preferred embodiment of the present invention.

EXAMPLE 1

10-(3'-chloropropyl)-phenoxazine. To a suspension of sodium amide (1.72 g) in 100 ml of liquid ammonia, 7g (0.038 mol) of phenoxazine was added. After stirring for 30 minutes, 6.3 g (0.04 mol., 3.96 mL) of 1-bromo-3-chloropropane was added slowly with constant stirring. After one more hour, ammonia was allowed to evaporate and solid ice pieces were added carefully followed by cold water. When the reaction ceased, the mixture was extracted three times with ether. The ether solution was washed three times with water, dried over anhydrous sodium sulfate and evaporated. The residue was chromatographed on silica gel. Petroleum ether-ethylacetate (9 mL + 3 mL) eluted the pure title compound (7.94 g) as white crystals. VU-λ_{max} (MeOH) 218, 238 and 321 nm; IR (KBr) 3070, 2860, 1630, 1490, 1380, 1275, 920, 815 and 740 cm^{-1}; ^{1}H-NMR (δ) 6.47-6.82 (m, 8H, ArH, H_1-H_4 and H_6-H_9), 2.11 (m, 2H, H_1), 3.63 (m, 2H, H_K), and 3.69 (m, 2H, H_m); ^{13}C-NMR (^{1}H decoupled) 111.23 (C_1 and C_9), 115.50 (C_4 and C_6), 121.07 (C_3 and C_7), 123.70 (C_2 and C_8), 133.03 ($C_{1'}$ and $C_{9'}$), 144.92 ($C_{4'}$ and $C_{6'}$), 27.82 (C_1), 41.09 (C_K) and 42.63 (C_m); EIMS (m/z) 259 (M^+).

EXAMPLE 2

10-(3'-diethylaminopropyl)phenoxazine. 1g (4.31 mmol) of the product of Example 1 was dissolved in 150 mL of anhydrous acetonitrile, and 1.5 g KI, 2.13 g K_2CO_3 and 1.6 mL (15.4 mmol) of N,N-diethylamine were added. The mixture was refluxed overnight until a substantial amount of product was formed (TLC, System B, R_f = 0.40). The reaction mixture was diluted with water and extracted with ether three times. The ether layer was washed with water and dried over anhydrous Na_2SO_4 and evaporated. The crude oil was subjected to column chromatography for purification. Ethylacetate-petroleum ether (50 mL + 50 mL) eluted the title compound as the free base as a colorless oil, which was dried and used for NMR studies. An ethereal solution of the free base was treated with an excess of tartaric acid to separate the hygroscopic tartrate salt (1.2 g). UV-λ_{max} (MeOH) 215, 238 and 320 nm; IR ($CHCl_3$) 3378, 2974, 2838, 1453, 1375, 1155, 973 and 722 cm^{-1}; ^{1}H-NMR ('δ') 6.51-6.80 (m, 8H, ArH, H_1-H_4 and H_6-H_9), 1.16 (t, 6H, H_c and H_d), 1.70 (m, 2H, H_l), 2.50 (q, 4H, H_a and H_b, J=7 Hz), 3.42-3.63 (m, 4H, H_k and H_m); ^{13}C-NMR 111.54 (C_1 and C_9), 115.49 (C_4 and C_6), 121.21 (C_3 and C_7), 123.85 (C_2 and C_8), 132.72 ($C_{1'}$ and $C_{9'}$), 144.95 ($C_{4'}$ and $C_{6'}$), 8.21 (C_c and C_d), 19.90 (C_l), 40.72 (C_a and C_b), 45.87 (C_m), and 48.50 (C_k); EIMS (m/z) 296 (M^+).

EXAMPLE 3

10-(3'-bishydroxyethylaminopropyl)phenoxazine. The procedure used for Example 2 was repeated with 1g, (4.31 mmol) of the product of Example 1, 1.5 g KI, and 1.62 g (15.4 mmol, 1.5 mL) of diethanolamine. Recrystallization of the solid in ethylacetate and petroleum ether gave (1.14 g) of the title compound in the pure form. UV-λ_{max} (MeOH) 218, 239, and 322 nm; IR (KBr) 3300, 2960, 2880, 1590, 1490, 1440, 1375, 1270, 1190, 1125, 1075, 1040, 890, 840, and 740 cm^{-1}; ^1H-NMR ('δ') 6.44-6.78 (m, 8H, ArH, H_1-H_4 and H_6-H_9), 1.71-1.82 (m, 2H, H_1), 2.54-2.61 (t, 4H, H_a and H_b, J = 6 Hz), 3.39 - 3.68 (m, 8H, H_x, H_c, and H_d and H_m), and 2.95 (s,H_e and H_f, disappearing on D_2O exchange); ^{13}C-NMR 111.37 (C_1 and C_9), 115.33 (C_4 and C_6), 120.80 (C_3 and C_7), 123.66 (C_2 and C_8), 133.25 ($C_{1'}$ and $C_{9'}$), 144.99 ($C_{4'}$ and $C_{6'}$), 22.42 (C_1), 41.83 (C_a and C_b), 52.38 (C_m), 55.91 (C_x) and 59.64 (C_c and C_d); EIMS (m/z) 328 (M^+).

EXAMPLE 4

10-(3'-N-morpholinopropyl)phenoxazine. The procedure used for Example 2 was repeated with 1g of the product of Example 1, 1.5 g KI, 2.0 g K_2CO_3 and 1.4 g (15.40 mmol, 1.34 mL) of morpholine. The oily residue was purified by column chromatography to give the title compound as a brown oil. An ethereal solution of the free base was treated with ethereal hydrochloride to give the hydro-chloride salt (1.07 g). UV-λ_{max} (MeOH) 216, 239, and 320 nm; IR (KBr) 3200, 1495, 1380, 1280, 1230, 1135, 1100, 1050, 1020, 980, 870, 830, 760 and 735 cm^{-1}; ^{1}H-NMR ('δ') 6.63-6.81 (m, 8H, ArH, H_1-H_4 and H_6-H_9), 1.78 (m, 2H, H_1), 2.40 (t, 4H, H_a and H_b, J = 12 Hz), 3.45-3.80 (m, 8H, K_x, H_m, H_c and H_d); ^{13}C-NMR 111.64 (C_1 and C_9), 115.80 (C_4 and C_6), 121.59 (C_3 and C_7), 123.91 (C_2 and C_8), 133.50 ($C_{1'}$ and $C_{9'}$), 145.11 ($C_{4'}$ and $C_{6'}$), 20.06 (C_1), 40.93 (C_a and C_b), 51.91 (C_m), 55.20 (C_x), and 63.50 (C_c and C_d); EIMS (m/z) 310 (M^+).

EXAMPLE 5

1 10-(3'-N-piperidinopropyl)phenoxazine. The procedure used for Example 2 was used with 1.12 g (4.31 mmol) of the product of Example 1, 1.5 g IH, 2.4 g K_2CO_3 and 1.5 g (17.62 mmol, 1.74 mL) of piperidine. The product was chromatographed on silica gel with petroleum ether-ethylacetate (1:1 by volume) to obtain the pure title compound in the form of an oil. By adding ethereal hydrochloride to the ether solution of the free base, the hydrochloride salt (1.15 g) was obtained. UV-λ_{max} (MeOH) 218, 238 and 320 nm; IR (KBr) 3300, 2940, 2680, 1595, 1495, 1385, 1275, 1160, 1050, 825 and 745 cm^{-1}; ^{1}H-NMR ('δ') 6.56-6.86 (m, 8H, ArH, H_1-H_4 and H_6-H_9), 1.53 (m, 6H, H_c, H_d and H_e), 2.30 (m, 2H, H_1), 2.56-2.67 (m, 4H, H_a and H_b), and 3.45-3.70 (m, 4H, H_x and H_m); ^{13}C-NMR 111.65 (C_1 and C_9), 115.62 (C_4 and C_6), 121.38 (C_3 and C_7), 123.88 (C_2 and C_8), 132.73 ($C_{1'}$ and $C_{9'}$), 144.98 ($C_{4'}$ and $C_{6'}$), 20.21 (C_e), 21.93 (C_c and C_d), 22.50 (C_1), 41.05 (C_a and C_b), 53.18 (C_m), and 54.62 (C_x); EIMS (m/z) 308 (M^+).

EXAMPLE 6

10-(3'-ß-hydroxyethylpiperazinopropyl) phenoxazine. The procedure used for Example 2 was repeated with 1 g (4.31 mmol) of the product of Example 1, 1.5 g KI, 2.12 g K_2CO_3 and 2 g (15.4 mmol, 1.9 mL) of ß-hydroxyethylpiperazine. The free base was recrystallized in petroleum ether-ether mixture (7:3 by volume) to give 1.16 g of the title compound. UV-λ_{max} (MeOH) 217, 239 and 322 nm; IR (KBr) 3060, 2820, 1630, 1595, 1495, 1385, 1270, 1160, 1070, 980, 850, 810 and 735 cm^{-1}; ^{1}H-NMR ('δ') 6.46-6.76 (m, 8H, ArH, H_1-H_4 and H_6-H_9), 1.74 (m, 2H, H_1), 2.33-2.80 (M, 12H, H_a and H_b, H_c and H_d, H_e and H_m), 2.79 (s, 1H, Hg, disappearing on D_2O exchange), 3.47-3.65 (m, 4H, H_x and H_f); ^{13}C-NMR 111.34 (C_1 and C_9), 115.24 (C_4 and C_6), 120.66 (C_3 and C_7), 123.50 (C_2 and C_8), 133.30 ($C_{1'}$ and $C_{9'}$), 144.83 ($C_{4'}$ and $C_{6'}$), 22.58 (C_1), 41.72 (C_m), 52.96 (C_a and C_b), 53.28 (C_c and C_d), 55.19 (C_x); 57.77 (C_e), and 59.34 (C_f); MS (m/z) 353 (M^+).

EXAMPLE 7

10-(3'-N-pyrrolidinopropyl)phenoxazine. The procedure used for Example 2 was repeated with 1g of the title product of Example 1, 1.5 g KI, 2g K_2CO_3 and 1.1g (15.5 mmol, 1.3 mL) of pyrrolidine. The product was purified by column chromatography and the oil was converted into the hydrochloride salt (1.02g). UV-λ_{max} (MeOH) 217, 239, and 319 nm; IR (KBr) 3300, 2660, 1590, 1490, 1375, 1270, 1130, 920, 820 and 745 cm^{-1}; ^{1}H-NMR ('δ') 6.46-6.77 (m, 8H, ArH, H_1-H_4 and H_6-H_9), 2.01-2.17 (t, 4H, H_c and H_d, J = 13 Hz), 2.21 (m, 2H, H_1), 3.06-3.14 (t, 4H, H_a and H_b), and 3.60-3.67 (m, 4H, H_k and H_m); ^{13}C-NMR 111.60 (C_1 and C_9), 115.66 (C_4 and C_6), 121.40 (C_3 and C_7), 123.85 (C_2 and C_8), 132.73 ($C_{1'}$ and $C_{9'}$), 144.98 ($C_{4'}$ and $C_{6'}$), 22.25 (C_c and C_d), 23.30 (C_1), 40.90 (C_a and C_b), 52.80 (C_m), and 53.63 (C_k); MS (m/z) 294 (M^+).

EXAMPLE 8

10-(4'-chlorobutyl)phenoxazine, (8.4 g) in the pure form was prepared following the procedure used for Example 1 with 7g phenoxazine, 1.63 g sodium amide and 4.36 mL of 1-bromo-4-chlorobutane (0.038 mol) to produce the title compound. UV-λ_{max} (MeOH) 200, 212, 238, and 320 nm; IR (KBr) 3060, 2980, 1630, 1590, 1495, 1380, 1280, 1130, 915, 840 and 730 cm^{-1}; ^{1}H-NMR ('δ') 6.36-6.74 (m, 8H, ArH, H_1-H_4 and H_6-H_9), 1.75 (broad, 4H, H_l and H_m), and 3.38-3.50 (m, 4H, H_k and H_n), ^{13}C-NMR 111.43 (C_1 and C_9), 115.53 (C_4 and C_6), 121.01 (C_3 and C_7), 123.83 (C_2 and C_8), 133.27 ($C_{1'}$ and $C_{9'}$), 145.10 ($C_{4'}$ and $C_{6'}$), 22.60 (C_m), 29.87 (C_l), 43.27 (C_k), and 44.61 (C_n); EIMS (m/z) 273 (M^+).

EXAMPLE 9

10-(4'-diethylaminobutyl)phenoxazine. The procedure used for Example 2 was followed with 1g (3.65 mmol) of the product of Example 8, 1.5g KI, 2g K_2CO_3 and 1.07 g (14.63 mmol, 1.5 mL) of N,N-diethylamine to obtain the indicated product. The oily product was chromato-graphed on the silica gel with CH_3OH-$CHCl_3$ (3:1) and the hydrochloride salt (.076g) was obtained in the pure form. UV-λ_{max} (MeOH) 201, 213, 239 and 320 nm; IR (KBr) 3300, 2940, 1590, 1495, 1380, 1270, 1130, 1040, 925 and 750 cm^{-1}; ^1H-NMR ('δ') 6.47-6.80 (m, 8H, ArH, H_1-H_4 and H_6-H_9), 1.33 (broad, 6H, H_c and H_d), 1.66-1.91 (m, 4H, H_l and H_m), 3.05 (very broad, 6H, H_a, H_b and H_n), and 3.50 (m, 2H, H_x); ^{13}C-NMR 111.51 (C_1 and C_9), 115.31 (C_4 and C_6), 120.99 (C_3 and C_7), 123.75 (C_2 and C_8), 132.78 ($C_{1'}$ and $C_{9'}$), 144.78 ($C_{4'}$ and $C_{6'}$), 8.54 (C_c and C_d), 21.02 (C_m), 22.46 (C_l), 43.05 (C_a and C_b), 46.50 (C_n), and 51.26 (C_x); MS (m/z) 310 (M^+).

EXAMPLE 10

10-(4'-bishydroxyethylaminobutyl) phenoxazine, as its hydrochloride salt (1.11g) was obtained by following the procedure of Example 3 with 1g of the product of Example 8, 1.5g KI and 1.54 g (14.65 mmol, 1.4 mL) of N,N-diethanolamine followed by column chromato-graphy. UV-λ_{max} (MeOH) 204, 210, 238 and 321 nm; IR (KBr) 3280, 2850, 1630, 1590, 1490, 1375, 1270, 1135, 1095, 1065, 1045, 1020, 925, 890, 845, and 740 cm^{-1}; ^{1}H-NMR ('δ') 6.52-6.84 (m, 8H, ArH, H_1-H_4 and H_6-H_9), 1.70-1.98 (m, 4H, H_1, and H_m), 3.35-3.57 (broad, 10H, H_a, H_b, H_n, H_x, H_e and H_f), 3.95 (t, 4H, H_c and H_d; J = 7 Hz), and 10.3 (H^+); ^{13}C-NMR 110.53 (C_1 and C_9), 114.17 (C_4 and C_6), 119.83 (C_3 and C_7), 122.76 (C_2 and C_8), 131.85 ($C_{1'}$ and $C_{9'}$), 143.60 ($C_{4'}$ and $C_{6'}$), 19.98 (C_m), 21.10 (C_1), 42.06 (C_n), 52.92 (C_a and C_b), 54.78 (C_x), and 54.96 (C_c and C_d); EIMS (m/z) 342 (M^+).

EXAMPLE 11

10-(4'-N-morpholinobutyl)phenoxazine. The procedure used for Example 4 was repeated with 1 g of the product of Example 8, 1.5g KI, 2g of K_2CO_3 and 1.273 g (14.61 mmol, 1.3 mL) of morpholine. The product was recrystallized in ether-petroleum ether mixture (3:1) to give the title compound (0.95g). UV-λ_{max} 202, 213, 239, and 321 nm; IR (KBr) 2960, 2810, 1630, 1595, 1495, 1380, 1295, 1220, 1130, 1070, 1010, 970, 920, 870, 855, 825, 765 and 745 cm^{-1}; ^{1}H-NMR ('δ') 6.53-7.29 (m, 8H, ArH, H_1-H_4 and H_6-H_9), 1.61-1.74 (m, 4H, H_1 and H_m), 2.40-2.50 (m, 6H, H_a, H_b, and H_n), 3.49 (m, 2H, H_x), and 3.49-3.78 (t, 4H, H_c and H_d, J = 12 Hz); ^{13}C-NMR 111.28 (C_1 and C_9), 115.28 (C_4 and C_6), 120.67 (C_3 and C_7), 123.52 (C_2 and C_8), 133.30 ($C_{1'}$ and $C_{9'}$), 144.99 ($C_{4'}$ and $C_{6'}$), 22.34 (C_m), 23.50 (C_1), 43.63 (C_n), 53.67 (C_a and C_b), 57.91 (C_x), and 66.97 (C_c and C_d); EIMS (m/z) 324 (M^+).

EXAMPLE 12

10-(4'-N-piperidinobutyl)phenoxazine. 1g of the product of Example 8, 1.5g of KI, 2g K_2CO_3 and 1.45g (17.03 mmol, 1.5 mL) of piperidine were refluxed and processed according to the procedure used for Example 10. Purification by column chromatography afforded the free amine as a brown oil which was converted into the hydrochloride salt (1.18 g). UV-λ_{max} 203, 210, 238, and 320 nm; IR (KBr) 3320, 2940, 1625, 1590, 1490, 1380, 1270, 1130, 1060, 955, 840, 820, and 730 cm^{-1}; ^{1}H-NMR ('δ') 6.42-6.81 (m, 8H, ArH, H_1-H_4 and H_6-H_9), 1.44-1.82 (m, 6H, H_c, H_d and H_e), 1.98-21.8 (m, H_l and H_m), 2.70-2.97 (m, 4H, H_a and H_b), 3.39-3.45 (m, 4H, H_x and H_n) and 11.54 (H^+); ^{13}C-NMR 111.42 (C_1 and C_9), 115.32 (C_4 and C_6), 120.98 (C_3 and C_7), 123.71 (C_2 and C_8), 132.78 ($C_{1'}$ and $C_{9'}$), 144.73 ($C_{4'}$ and $C_{6'}$), 20.96 (C_e), 21.79 (C_c and C_d), 22.48 (C_l and C_m), 43.08 (C_a and C_b), 52.91 (C_n), and 56.70 (C_x); EIMS (m/z) 322 (M^+).

EXAMPLE 13

10-(4'-ß-hydroxyethylpiperazinobutyl) phenoxazine. The procedure used for Example 6 was repeated with 1 g of the product of Example 8, 1.5g KI, and 1.9g (14.6 mmol, 1.8 mL) of ß-hydroxyethylpiperazine. The oily residue was treated with 500 µl of ethylacetate first and then with petroleum ether (20 mL), when a white crystalline solid separated out. The solid was recrystallized to give the pure title compound (1.21g). UV-λ_{max} (MeOH) 202, 239, and 320 nm; IR (KBr) 3060, 2940, 2860, 1590, 1495, 1380, 1225, 1135, 1020, 1005, 935, 880, 830, 780, and 740 cm^{-1}; ^1H-NMR ('δ') 6.46-6.75 (m, 8H, ArH, H_1-H_4 and H_6 and H_9), 1.58 (broad, 4H, H_l and H_m), 2.36-2.51 (m, 12H, H_a, H_b, H_c, H_d, H_e and H_n), 3.42 (broad, 3H, H_k, and H_g), and 3.58-3.63 (t, 2H, H_f, J = 7 Hz); ^{13}C-NMR 111.39 (C_1 and C_9), 115.26 (C_4 and C_6), 120.64 (C_3 and C_7), 123.61 (C_2 and C_8), 133.30 ($C_{1.}$ and $C_{9.}$), 144.95 ($C_{4.}$ and $C_{6.}$), 22.28 (C_l and C_m), 23.72 (C_n), 43.60 (C_a and C_b), 53.11 (C_c and C_d), 57.38 (C_k), 57.96 (C_e) and 59.76 (C_f); EIMS (m/z) 367 (M^+).

EXAMPLE 14

10-(4'-N-pyrrolidinobutyl)phenoxazine. The experimental steps used for Example 2 were repeated using 1g of the product of Example 8, 1.5g KI, 2g K_2CO_3 and 1.04g (14.6 mmol, 1.22 mL) of pyrrolidine as reactants. The product was chromatographed on silica gel with $CHCl_3$-MeOH (1:1) to give the free amine as a brown oil. An ether solution of this oil was treated with ethereal hydrogen chloride to secure the pure (0.9g) hydrochloride salt. UV-λ_{max} (MeOH) 205, 211, 238 and 320 nm; IR (KBr) 3060, 2840, 1590, 1495, 1380, 1295, 1270, 1160, 1090, 1045, 915, 840, 830, 795, and 740 cm^{-1}; ^1H-NMR ('δ') 6.43-6.79 (m, 8H, ArH, H_1-H_4 and H_6-H_9), 1.64-2.10 (m, 8H, H_1, H_m, H_c and H_d), 2.97-3.17 (m, 6H, H_a, H_b and H_n), 3.45-3.54 (m, 2H, H_x) and 10.10 (H^+); ^{13}C-NMR 111.43 (C_1 and C_9), 115.41 (C_4 and C_6), 121.01 (C_3 and C_7), 123.73 (C_2 and C_8), 132.89 ($C_{1'}$ and $C_{9'}$), 144.87 ($C_{4'}$ and $C_{6'}$), 22.47 (C_c and C_d), 23.27 (C_1 and C_m), 43.14 (C_a and C_b), 53.50 (C_n), and 54.91 (C_x); EIMS (m/z) 308 (M^+).

EXAMPLE 15

10-(chloroacetyl)phenoxazine. To a solution of 5g (0.03 mol) of phenoxazine dissolved in 100 mL anhydrous acetonitrile containing 10 mL of anhydrous ether, was added dropwise 7 mL (9.926 g, 0.088 mol) of chloroacetyl-chloride with constant stirring. The reaction mixture was stirred at room temperature for 5H when white crystalline solid separated out (TLC, system A, R_f=0.030). The crystals were filtered, washed several times with petroleum ether-ether mixture (9:1) and dried under high vacuum to get 6.03g of the product. UV-λ_{max} (MeOH) 218, 249, and 287 nm; IR (KBr) 3070, 1675, 1580, 1480, 1410, 1350, 1260, 1210, 1115, 1040, 860, 815, 750 and 660 cm^{-1}; ^{1}H-NMR ('δ') 7.55-7.61 (m, 2H, ArH, H_1 and H_9), 7.12-7.25 (m, 6H, ArH, H_2-H_4 and H_6-H_8), 4.32 (s, 2H, H_1); ^{13}C-NMR 110.04 (C_1 and C_9), 117.11 (C_4 and C_6), 123.75 (C_3 and C_7), 124.32 (C_2 and C_8), 127.60 ($C_{1'}$ and $C_{9'}$), 150.95 ($C_{4'}$ and $C_{6'}$), 41.51 (C_1), and 170 (C_x); EIMS (m/z) 259 (M^+).

EXAMPLE 16

10-(diethylaminoacetyl)phenoxazine. 1g (3.9 mmol) of the product of Example 15 was dissolved in 150 mL of anhydrous acetonitrile and 1.5g of KI and 1.13 g (15.45 mmol, 1.6 mL) of N,N-diethylamine were added to it. The reaction mixture was refluxed for 1h when substantial amount of the product was formed (TLC, system B, R_f=0.40). The mixture was processed as in Example 2 to get a white crystalline solid which was further recrystallized in ethylacetate and petroleum ether mixture to get the pure compound (0.86g). UV-λ_{max} (MeOH) 220, 246, and 287 nm; IR (KBr) 2800, 1685, 1580, 1480, 1320, 1210, 1150, 1060, 1035, 940, 860, 810, 755 and 670 cm^{-1}; ^{1}H-NMR ('δ') 7.53-7.59 (m, 2H, ArH, H_1 and H_9), 7.05-7.20 (m, 6H, ArH, H_2-H_4 and H_6-H_8), 0.95 (t, 6H, H_c and H_d, J=7 Hz), 2.60 (q, 4H, H_a and H_b), and 3.55 (s, 2H, H_1); ^{13}C-NMR 116.79 (C_1 and C_9), 123.31 (C_4 and C_6), 125.02 (C_3 and C_7), 126.82 (C_2 and C_8), 129.62 ($C_{1'}$ and $C_{9'}$), 151.07 ($C_{4'}$ and $C_{6'}$), 12.08 (C_c and C_d), 47.04 (C_a and C_b), 54.99 (C_1), and 169.84 (C_x); MS (m/z) 296 (M^+).

EXAMPLE 17

1 10-(N-morpholinoacetyl)phenoxazine. The same procedure used for Example 16 was employed with 1g of the product of Example 15, 1.5g KI and 1.347g (16 mmol, 1.4 mL) of morpholine. The solid product was recrystallized in a mixture of ethylacetate, petroleum ether and ether and the free base was converted into hydrochloride salt (1.07g) using ethereal hydrochloride. UV-λ_{max} 213, 246, and 287 nm; IR (KBr) 2980, 2860, 1690, 1485, 1440, 1355, 1270, 1180, 1120, 1070, 1005, 900, 870, 855, 760 and 640 cm^{-1}; ^{1}H-NMR ('δ') 7.60 (broad, 2H, ArH, H_1 and H_9), 7.12-7.34 (m, 6H, ArH, H_2-H_4 and H_6-H_8), 2.40-2.60 (t, 64, H_a and H_b, J=12 Hz), 3.35 (s, 2H, H_1) and 3.50-3.70 (t, 4H, H_c and H_d); ^{13}C-NMR 117.03 (C_1 and C_9), 123.90 (C_4 and C_6), 124.98 (C_3 and C_7), 126.95 (C_2 and C_8), 127.91 ($C_{1\cdot}$ and $C_{9\cdot}$), 150.54 ($C_{4\cdot}$ and $C_{6\cdot}$), 52.41 (C_a and C_b), 57.01 (C_1), 63.23 (C_c and C_d), and 163.40 (C_k); MS (m/z) 310 (M^+).

EXAMPLE 18

1 10-(N-piperidinoacetyl)phenoxazine. The method employed for Example 17 was used with 1g of the product of Example 15, 1.5g KI and 1.31g (15.4 mmol, 1.52 mL) of piperidine to get 0.95g of the title compound. UV-λ_{max} (MeOH) 218, 246 and 287 nm; IR (KBr) 2960, 1670, 1610, 1580, 1480, 1370, 1330, 1260, 1190, 1120, 1040, 940, 890, 855, 810, 765, and 655 cm^{-1}; ^1H-NMR ('δ') 7.57-7.61 (m, 2H, ArH, H_1 and H_9), 7.12-7.16 (m, 6H, ArH, H_2-H_4 and H_6-H_8), 1.51 (very broad, 6H, H_c, H_d and H_e), 2.44 (m, 4H, H_a and H_b) and 3.34 (s, 2H, H_1); ^{13}C-NMR 116.72 (C_1 and C_9), 123.28 (C_4 and C_6), 124.97 (C_3 and C_7), 126.79 (C_2 and C_8), 129.48 ($C_{1'}$ and $C_{9'}$), 151.01 ($C_{4'}$ and C_6 '), 23.92 (C_e), 25.93 (C_c and C_d), 54.15 (C_a and C_b), 60.80 (C_1), and 168.92 (C_k); EIMS (m/z) 308 (M^+).

EXAMPLE 19

10-(ß-hydroxyethylpiperazinoacetyl) phenoxazine. The procedure used for Example 17 was repeated with 1g of the product of Example 15, 1.5g KI and 2g (15.4 mmol, 1.9 mL) of ß-hydroxyethylpiperazine. Recrystallization of the white solid yielded 1.17 g of the title compound. UV_{max} (MeOH) 213, 246 and 287 nm; IR (KBr) 3200, 2940, 1685, 1665, 1480, 1265, 1190, 1160, 945, 855, 765 and 640 cm^{-1}; ^{1}H-NMR ('δ') 7.53-7.58 (m, 2H, ArH, H_1 and H_9), 7.08-7.25 (m, 6H, ArH, H_2-H_4 and H_6-H_8), 2.48 (m, 10H, H_a, H_b, H_c, H_d and H_e), 2.70 (s, 1H, H_g, disappearing on D_2O exchange), 3.39 (s, 2H, H_1) and 3.60 (t, 2H, H_f, J=7 Hz); ^{13}C-NMR 116.85 (C_1 and C_9), 123.34 (C_4 and C_6), 124.86 (C_3 and C_7), 126.99 (C_2 and C_8), 129.25 ($C_{1'}$ and $C_{9'}$), 151.04 ($C_{4'}$ and $C_{6'}$), 52.70 (C_a and C_b), 52.90 (C_c and C_d), 57.70 (C_g), 59.23 (C_1), 59.80 (C_f), and 168.43 (C_x); EIMS (m/z) 353 (M^+).

EXAMPLE 20

10-(N-pyrrolidinoacetyl)phenoxazine. The experimental procedure used for Example 17 was employed with 1g of the product of Example 15, 1.5g KI and 1.095g (15.4 mmol, 1.3 mL) of pyrrolidine. Purification by recrystallization afforded 1.02 g of the title compound. UV-λ_{max} (MeOH) 214, 240, and 286 nm; IR (KBr) 2980, 2820, 1695, 1670, 1480, 1455, 1340, 1270, 1180, 1100, 1040, 985, 905, 855, 755 and 640 cm^{-1}; ^{1}H-NMR ('δ') 7.58-7.63 (m, 2H, ArH, H_1 and H_9), 7.07-7.18 (m, 6H, ArH, H_2-H_4 and H_6-H_8), 1.77 (t, 4H, H_c and H_d, J=7 Hz), 2.64 (t, 4H, H_a and H_b) and 3.51 (s, 2H, H_1); ^{13}C-NMR 116.80 (C_1 and C_9), 123.33 (C_4 and C_6), 125.06 (C_3 and C_7), 126.85 (C_2 and C_8), 129.28 ($C_{1'}$ and $C_{9'}$), 151.00 ($C_{4'}$ and $C_{6'}$), 23.73 (C_c and C_d), 53.83 (C_a and C_b), 57.24 (C_1), and 168.92 (C_x); EIMS (m/z) 294 (M^+).

EXAMPLE 21

10-(trifluoroacetyl)phenoxazine. To a solution of 200 mg of phenoxazine in 10 mL anhydrous chloroform and 4 mL anhydrous ether, was added 50 µl of (0.7435g, 3.54 mmol) trifluoroacetic anhydride. The resulting mixture was stirred at room temperature for 8 hours. The formation of the product was monitored by TLC (system A). The product solution was then extracted with chloroform and evaporated. The residue was subjected to column chromatography which afforded the pure title compound. UV-λ_{max} (MeOH) 212, 238, and 252 nm: IR (KBr) 3375, 1695, 1580, 1480, 1455, 1390, 1290, 1170, 1110, 1030, 965, 890, 850, 800, 760, 730, and 670 cm^{-1}; ^{1}H-NMR ('δ') 7.57-7.61 (m, 2H, ArH, H_1 and H_9), 7.14-7.32 (m, 6H, ArH, H_2-H_4 and H_6-H_8); ^{13}-C-NMR 117.20 (C_1 and C_9), 123.83 (C_4 and C_6), 124.34 (C_3 and C_7), 128.34 (C_2 and C_8), 151.04 ($C_{1'}$ and $C_{9'}$, and $C_{4'}$ and $C_{6'}$), and >200 ppm (C_x and C_1); EIMS (m/z) 279 (M^+).

TABLE I

PHYSICAL PROPERTIES OF N-(ALKYLAMINO) OR N-ACYLAMINO DERIVATIVES OF PHENOXAZINE		
Product Of Example No.	Yield, %	mp, °C
1	80	53
2	70	ND
3	90	83-84
4	80	198*
5	70	202*
6	85	108
7	75	158-159*
8	80	46
9	60	127*
10	80	115*
11	80	89 187*
12	90	190*
13	90	114
14	70	170*
15	85	143-144
16	75	39
17	80	130*
18	80	110-111
19	85	70-71
20	80	96-98*
21	70	90
* - HCl salt		

The potentiating agent is preferably administered by infusion in solution in sterile water. The potentiating agents as hydrochloride salts can be dissolved in sterile water. The agents as bases can be solubilized in 1N hydrochloric acid, following which the solution is back titrated with sodium hydroxide to provide a final pH between 7 and 8.

Cytotoxic agents whose cytotoxicity would be potentiated by agents within the scope of this invention include VCR, VLB, doxorubicin, colchicine, actinomycin D, daunomycin, M-AMSA, and other anthracyclic compounds.

The potentiating agent is administered to tumor cells which are exposed to one or more cytotoxic agents. By "exposed" is meant that the cytotoxic agent has been administered simultaneously with the potentiating agent, and/or is administered subsequently to the administration of the potentiating agent, so long as at least some of the cytotoxic agent(s) is present in the tumor cell when the potentiating agent is present in the tumor cell. The cytotoxic agent should not be administered before the potentiating agent. Preferably, the cytotoxic agent is administered when the potentiating agent concentration reaches steady state during administration by infusion.

It will be recognized that the amount of potentiating agent to be administered will vary between hosts, between cytotoxic agents and between potentiating agents, but the effective amounts can readily be ascertained by those of ordinary skill in this field. As guidance one can refer to the data in Examples 22-24 as well as the following Table. In general, though, effective amounts to potentiate cytotoxic agents are

about 2000-3000 moles of potentiating agent per mole of VCR; about 1,000-2,000 moles of potentiating agent per mole of VLB; and about 25-35 moles of potentiating agent per mole of VP-16 (Etoposide). These values, and the corresponding values for any other cytotoxic agents, can readily be converted if desired into dosages per host body weight by calculation based on the dosages for the cytotoxic agent of interest. The <u>in vitro</u> techniques described herein can be employed to determine the effectiveness of any particular potentiating agent with any given cytotoxic agent or agents.

EXAMPLE 22

Table II below gives representative *in vivo* values of the molar ratios (shown below as "compound: (cytotoxic agent)") of potentiating agent to cytotoxic agent for compounds within the scope of this invention. Vincristine (VCR) was administered to mice at 3 mg/kg (3.25 µmol/kg); vinblastine (VLB) was at 5 mg/kg (5.5 µmol/kg); VP-16 (Etoposide) was at 50 mg/kg/day for 3 days (0.255 mmol/kg total). The compound number is the number of the example in which the potentiating agent was prepared.

TABLE II

Compound No.	Compound:VCR	Compound:VLB	Compound:VP-16
3	2345	1388	29.9
4	2483	1469	31.6
11	2375	1405	30.3
18	2498	1478	31.8

EXAMPLE 23

Evaluation of N-substituted Phenoxazines For Anti-MDR activity

A cloned line of human colon adenocarcinoma, GC_3/Cl^{31}, which is intrinsically resistant to VCR (≈ 4-fold relative to KB-3-1), was routinely grown at 37°C in antibiotic-free RPMI-1640 medium supplemented with 2 mM glutamine and 10% FBS (Hyclone Laboratories, Inc., Logan, UT) in a humidified atmosphere of 5% CO_2 and 95% air. Human epidermoid carcinoma KB-3-1 cells and a colchicine selected MDR variant, $KBCh^R$-8-5, were obtained which was cross-resistant to VCR (45-fold) and VLB (6.3-fold); it was grown in monolayer culture at 37°C in DMEM with 10% FBS and L-glutamine in a humidified atmosphere of 10% CO_2 in air. The resistance of the $KBCh^R$-8-5 cells was maintained by culturing them with colchicine (10 ng/ml).

Then, 2 mL of cell suspensions (2 x 10^6) were plated in 35 x 10 mm style "easy grip" culture dishes (Becton Dickinson Co., Lincoln Park, NJ). Cells were allowed to attach to plastic overnight at 37°C. Medium was aspirated and cells were washed with (2 x 2 mL) physiologic tris (PT) buffer. Monolayers were incubated at room temperature for 10 minutes in PT buffer prior to aspiration and adding 1 mL of serum-free RPMI-1640 Hepes buffer (10.4g RPMI-1640 medium in IL of 25 mM Hepes, pH 7.4) containing 70.4 nm [^{3}H] VCR (sp.act. 7.1 ci/mmol) or 49.5 nm [^{3}H] VLB (sp. act. 10.1 Ci/mmol) with or without a compound of Examples 1-21 (100 µM) or VRP dissolved in H_2O dissolved in DMSO (final culture concentration <0.1% DMSO). After 2h of incubation at room temperature, medium was rapidly aspirated to

terminate drug accumulation, and monolayers were washed four times with ice-cold PBS (g/L: NaCL 8.0; $Na_2HPO_4.12H_2O$, 2.9; KCl 0.2; KH_2PO_4, 0.2) and drained. To each dish, 1 ml of trypsin-EDTA (0.05% trypsin, 0.53 mM EDTA) was added. After 1 minute, monolayers were triturated to give a uniform suspension of cells, and radioactivity in 0.75 ml was determined by scintillation counting. Cell number per dish was determined on 200 µl of suspension using the method of Butler, and amounts of intracellular VCR or VLB were determined. The results are set forth in Table III, in which the compound number is the number of the Example in which the compound (or "modulator" or "potentiating agent") was prepared.

TABLE III

EFFECTS OF N-SUBSTITUTED PHENOXAZINES ON MDR ACTIVITY

Modulator Compound Number	Vinca Accumulation [a] (% control) KB Ch^R-8-5 Cells VCR	VLB	GC_3/Cl Cells VCR	VLB
1	454	342	846	570
2	546	2123	439	1025
3	473	1666	464	1070
4	742	1717	634	960
5	435	1227	282	633
6	343	824	368	879
7	408	969	250	757
8	398	792	317	361
9	211	697	325	737
10	92	403	382	1165
11	702	2684	477	1175
12	196	1071	416	1121
13	91	188	543	1340
14	198	477	412	1315
15	138	236	171	284
16	184	953	160	305
17	290	674	213	298
18	326	2023	177	446
19	280	776	157	426
20	188	776	151	296
21	415	827	230	222
Verapamil	402	1124	178	238

[a] $\dfrac{\text{vinca uptake with modulator}}{\text{vinca uptake without modulator}} \times 100$

[b] Compounds were tested at 100μM. All values represent the mean of two separate experiments with a SD of less than 10% of the mean; each experiment was done in triplicate.

EXAMPLE 24

Evaluation of N-substituted Phenoxazines Cytotoxicity To Tumor Cells

The KBCh^{R}-8-5 cells were plated in triplicate at a density of 1000 cells per well and GC_3 at 3000 cells per well in Falcon 6-well flat-bottom tissue culture plates (Becton Dickinson Co., Lincoln Park, NJ). After 24h, incubation medium was replaced with 3 mL of fresh medium containing compounds 1-4 or 10-14 or 18 at concentrations ranging from 1-100 µm (final culture concentration, 0.1% DMSO), and cells were incubated at 37°C for a further 7 days. The medium was aspirated and cells were washed once with 2 mL of 0.9% saline and dried overnight. Colonies were stained with 1 mL of 0.1% crystal violet followed by washing twice with distilled water and were counted using an automated ARTEK Model 880 colony counter. The IC_{50} values were determined from concentration-percent-cell-survival curves and were defined as the concentrations of phenoxazines required for 50% reduction in colonies compared to controls. The results of these measurements are set forth in Table IV.

TABLE IV

CYTOTOXICITY OF N-SUBSTITUTED PHENOXAZINES		
	IC_{50},[a] μM	
Compound Number	$KBCh^R$-8-5	GC_3/CL
1	57	83.00
2	15	ND
3	38	37
4	73	40
10	<10	16
11	18	27
12	<10	7
13	<10	7
14	<10	8
18	73	ND

[a] IC_{50} is the concentration required to produce 50% reduction in clonogenic survivial of GC_3/Cl and $KBCH^R$-8-5 cells under the conditions described in Example 23.

EXAMPLE 25

Effect Of N-substituted Phenoxazines On In Vitro Cytotoxicity Of VLB And VCR

Tumor cells were treated with graded concentrations of VCR and VLB in the absence or presence of nontoxic concentrations of the products of Examples 1, 3, 4 and 18. The plates were then transferred to a CO_2 incubator and, after further incubation for 7 days at 37°C, colonies were enumerated as described in Example 23. The results are set forth in Table V.

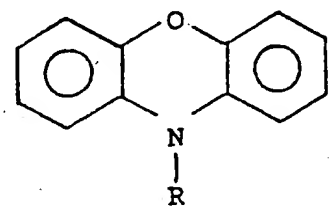
TABLE V

Potentiation Of Cytotoxicity Of Vincristine And Vinblastine By N-substituted Phenoxazines Against GC_3/Cl And $KBCh^R$-8-5 Cells					
		IC_{50} Values, nM			
		KB Ch^R-8-5 Cells		GC_3/Cl Cells	
Compound Number	Concentration of Modulator[a] (μM)	VCR	VLB	VCR	VLB
no modulator	-	32.0	20.0	27.0	7.4
1	50	-	-	9.0	-
3	25	-	2.7	-	2.0
4	25	1.2	1.6	0.85	2.0
18	49	-	2.3	-	2.2

[a] IC_{50} concentration of modulator

WHAT IS CLAIMED IS:

1. A method of potentiating the cytotoxicity of an agent cytotoxic to a tumor cell, comprising administering to said tumor cell, while it is exposed to said cytotoxic agent, a potentiating agent in an amount effective to potentiate the cytotoxicity of said cytotoxic agent to said cell, wherein said potentiating agent comprises a compound of the formula (1):



(1)

or a pharmacologically acceptable salt thereof, wherein R is -H or $-[C(O)]_a-(CH_2)_b-A$; wherein a is 0 or 1 and b is an integer from 0 to 6, provided that a and b are not both zero; and

A is selected from the group consisting of

$-NR_1R_2$ wherein R_1 and R_2 are independently alkyl having 1 to 4 carbon atoms, and either or both of R_1 and R_2 are optionally substituted with -OH;

```
      X
    /   \
 -N       Z   wherein X and Y are independently
    \   /
      Y
```

alkylene having 1 to 4 carbon atoms, and Z is -O-, $-N(R_3)$-or $-CH(R_4)$-, wherein R_3 is hydrogen or alkyl having 1 to 4 carbon atoms optionally substituted with a hydroxyl group, and wherein R_4 is hydrogen or alkyl having 1 to 4 carbon atoms optionally substituted with a hydroxyl group;

halide; and trihalomethyl.

2. The method of Claim 1 wherein said tumor cell is present in a living host.

3. The method of Claim 1 wherein said cytotoxic agent is selected from the group consisting of vincristine, vinblastine, etoposide, doxorubicin, colchicine, actinomycin D, daunomycin, m-AMSA, and mixtures thereof.

4. The method of Claim 1 wherein said tumor cell exhibits multiple drug resistance.

5. The method of Claim 1 wherein a is zero; b is 3 or 4; R_1 and R_2 are independently selected from the group consisting of ethyl, propyl, ω-hydroxyethyl, and ω-hydroxypropyl; X and Y are each independently selected from the group consisting of $-CH_2-$ and $-CH_2CH_2-$; and R_3 and R_4 are independently selected from the group consisting of -H, ethyl, propyl, ω-hydroxyethyl, and ω-hydroxypropyl.

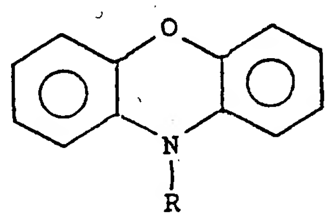
6. The method of Claim 5 wherein said potentiating agent is 10-(3'-chloropropyl)-phenoxazine, 10-(3'-diethylaminopropyl)-phenoxazine, 10-(3'-bishydroxyethylaminopropyl)-phenoxazine, 10-(3'-N-morpholinopropyl)-phenoxazine, 10-(3'-N-piperidinopropyl)-phenoxazine, 10-(3'-ß-hydroxyethylpiperazinopropyl)-phenoxazine, (10-(3'-N-pyrrolidinopropyl)-phenoxazine, 10-(4'-chlorobutyl)-phenoxazine, 10-(4'-diethylaminobutyl)-phenoxazine, 10-(4'-bishydroxyethylaminobutyl)-phenoxazine, 10-(4'-N-morpholinobutyl)-phenoxazine,10-(4'-piperidinobutyl)-phenoxazine, 10-(4'-ß-hydroxyethylpiperazinobutyl)-phenoxazine, 10-(4'-N-pyrrolidinobutyl)-phenoxazine or pharmacologically acceptable salts thereof.

7. The method of Claim 1 wherein a is 1.

8. The method of Claim 7 wherein b is 1 or 2; R_1 and R_2 are independently selected from the group consisting of ethyl, propyl, ω-hydroxyethyl, and ω-hydroxypropyl; X and Y are each independently selected from the group consisting of $-CH_2-$ and $-CH_2CH_2-$; and R_3 and R_4 are independently selected from the group consisting of -H, ethyl, propyl, ω-hydroxyethyl, and ω-hydroxypropyl.

9. The method of Claim 8 wherein said potentiating agent is 10-(chloroacetyl)-phenoxazine, 10-(diethylaminoacetyl)-phenoxazine, 10-(N-morpholinoacetyl)-phenoxazine, 10-(N-piperidinoacetyl)-phenoxazine, 10-(ß-hydroxyethylpiperazinoacetyl)-phenoxazine, 10-(N-pyrrolidinoacetyl)-phenoxazine, 10-(trifluoroacetyl)-phenoxazine or pharmacologically acceptable salts thereof.

10. A composition comprising a cytotoxic agent toxic to tumor cells, and a potentiating agent which potentiates the cytotoxicity of said cytotoxic agent, wherein said potentiating agent comprises a compound of the formula (1)



(1)

or a pharmacologically acceptable salt thereof, wherein R is -H or $-[C(O)]_a-(CH_2)_b-A$; wherein a is 0 or 1 and b is an integer from 0 to 6, provided that a and b are not both zero; and

A is selected from the group consisting of

$-NR_1R_2$ wherein R_1 and R_2 are independently alkyl having 1 to 4 carbon atoms, and either or both of R_1 and R_2 are optionally substituted with -OH;

```
        X
      /   \
   -N       Z
      \   /
        Y
```

wherein X and Y are independently alkylene having 1 to 4 carbon atoms, and Z is -O-, -$N(R_3)$-or -$CH(R_4)$-, wherein R_3 is hydrogen or alkyl having 1 to 4 carbon atoms optionally substituted with a hydroxyl group, and wherein R_4 is hydrogen or alkyl having 1 to 4 carbon atoms optionally substituted with a hydroxyl group;

halide; and trihalomethyl;

wherein said cytotoxic agent and potentiating agent are present in amounts effective to render the composition cytotoxic to tumor cells.

11. The composition of Claim 10 wherein said cytotoxic agent is selected from the group consisting of vincristine, vinblastine, etoposide, doxorubicin, colchicine, actinomycin D, daunomycin, m-AMSA, and mixtures thereof.

12. The composition of Claim 10 wherein a is zero; b is 3 or 4; R_1 and R_2 are independently selected from the group consisting of ethyl, propyl, ω-hydroxyethyl, and ω-hydroxypropyl; X and Y are each independently selected from the group consisting of -CH_2- and -CH_2CH_2-; and R_3 and R_4 are independently selected from the group consisting of -H, ethyl, propyl, ω-hydroxyethyl, and ω-hydroxypropyl.

13. The composition of Claim 12 wherein said potentiating agent is 10-(3'-chloropropyl)-phenoxazine,

10-(3'-diethylaminopropyl)-phenoxazine, 10-(3'-bishydroxyethylaminopropyl)-phenoxazine, 10-(3'-N-morpholinopropyl)-phenoxazine, 10-(3'-N-piperidinopropyl)-phenoxazine, 10-(3'-ß-hydroxyethylpiperazinopropyl)-phenoxazine, (10-(3'-N-pyrrolidinopropyl)-phenoxazine, 10-(4'-chlorobutyl)-phenoxazine, 10-(4'-diethylaminobutyl)-phenoxazine, 10-(4'-bishydroxyethylaminobutyl)-phenoxazine, 10-(4'-N-morpholinobutyl)-phenoxazine,10-(4'-piperidinobutyl)-phenoxazine, 10-(4'-ß-hydroxyethylpiperazinobutyl)-phenoxazine, 10-(4'-N-pyrrolidinobutyl)-phenoxazine or pharmacologically acceptable salts thereof.

14. The composition of Claim 10 wherein a is 1; b is 1 or 2; R_1 and R_2 are independently selected from the group consisting of ethyl, propyl, ω-hydroxyethyl, and ω-hydroxypropyl; wherein X and Y are each independently selected from the group consisting of $-CH_2-$ and $-CH_2CH_2-$; and R_3 and R_4 are independently selected from the group consisting of -H, ethyl, propyl, ω-hydroxyethyl, and ω-hydroxypropyl.

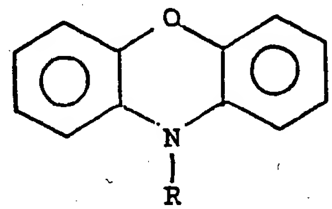
15. The composition of Claim 14 wherein said potentiating agent is 10-(chloroacetyl)-phenoxazine, 10-(diethylaminoacetyl)-phenoxazine, 10-(N-morpholinoacetyl)-phenoxazine, 10-(N-piperidinoacetyl)-phenoxazine, 10-(ß-hydroxyethylpiperazinoacetyl)-phenoxazine, 10-(N-pyrrolidinoacetyl)-phenoxazine, 10-(trifluoroacetyl)-phenoxazine or pharmacologically acceptable salts thereof.

16. A method of killing a tumor cell which comprises administering to said cell a composition according to Claim 10 in an amount effective to kill said cell.

17. Th method of Claim 16 wherein said tumor cell is present in a living host.

18. The method of Claim 16 wherein said tumor cell exhibits multiple drug resistance.

19. A compound of the formula (1)



(1)

and pharmacologically acceptable salts thereof, wherein R is $-[C(O)]_a-(CH_2)_b-A$; wherein a is 0 or 1 and b is an integer from 0 to 6, provided that a and b are not both zero; and

A is selected from the group consisting of

$-NR_1R_2$ wherein R_1 and R_2 are independently alkyl having 1 to 4 carbon atoms, and either or both of R_1 and R_2 are optionally substituted with -OH;

$$-N\begin{matrix} / & X & \backslash \\ & & \\ \backslash & Y & / \end{matrix}Z$$

wherein X and Y are independently alkylene having 1 to 4 carbon atoms, and Z is -O-, -$N(R_3)$-or $-CH(R_4)-$, wherein R_3 is hydrogen or alkyl having 1 to 4 carbon atoms optionally substituted with a hydroxyl group, and wherein R_4 is hydrogen or alkyl having 1 to 4 carbon atoms optionally substituted with a hydroxyl group;

halide; and trihalomethyl.

20. A compound or salt according to Claim 19 wherein a is zero; b is 3 or 4; R_1 and R_2 are

independently selected from the group consisting of ethyl, propyl, ω-hydroxyethyl, and ω-hydroxypropyl; X and Y are each independently selected from the group consisting of $-CH_2-$ and $-CH_2CH_2-$; and R_3 and R_4 are independently selected from the group consisting of -H, ethyl, propyl, ω-hydroxyethyl, and ω-hydroxypropyl.

21. The compound according to Claim 20 which is 10-(3'-chloropropyl)-phenoxazine, 10-(3'-diethylaminopropyl)-phenoxazine, 10-(3'-bishydroxyethylaminopropyl)-phenoxazine, 10-(3'-N-morpholinopropyl)-phenoxazine, 10-(3'-N-piperidinopropyl)-phenoxazine, 10-(3'-β-hydroxyethylpiperazinopropyl)-phenoxazine, (10-(3'-N-pyrrolidinopropyl)-phenoxazine, 10-(4'-chlorobutyl)-phenoxazine, 10-(4'-diethylaminobutyl)-phenoxazine, 10-(4'-bishydroxyethylaminobutyl)-phenoxazine, 10-(4'-N-morpholinobutyl)-phenoxazine, 10-(4'-piperidinobutyl)-phenoxazine 10-(4'-β-hydroxyethylpiperazinobutyl)-phenoxazine, 10-(4'-N-pyrrolidinobutyl)-phenoxazine or pharmacologically acceptable salts thereof.

22. A compound or salt according to Claim 19 wherein a is 1; b is 1 or 2; R_1 and R_2 are independently selected from the group consisting of ethyl, propyl, ω-hydroxyethyl, and ω-hydroxypropyl; X and Y are each independently selected from the group consisting of $-CH_2-$ and $-CH_2CH_2-$; and R_3 and R_4 are independently selected from the group consisting of -H, ethyl, propyl, ω-hydroxyethyl, and ω-hydroxypropyl.

23. The compound according to Claim 22 which is 10-(chloroacetyl)-phenoxazine, 10-(diethylaminoacetyl)-phenoxazine, 10-(N-morpholinoacetyl)-phenoxazine, 10-(N-piperidinoacetyl)-

phenoxazine, 10-(β-hydroxyethylpiperazinoacetyl)-phenoxazine, 10-(N-pyrrolidinoacetyl)-phenoxazine, 10-(trifluoroacetyl)-phenoxazine or pharmacologically acceptable salts thereof.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 92/06681

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 A 61 K 31/535 C 07 D 265/38

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	Cancer Communications, vol. 2, no. 7, 1990, Pergamon Press, (US), K.N. THIMMAIAH et al.: "Structural determinants of phenoxazine type compounds required to modulate the accumulation of vinblastine and vincristine in multidrug-resistant cell lines", pages 249-259, see abstract; page 249; page 251, table 1; pages 257-258	1-4,10, 11,16-18

Y		5-9
	--- -/-	

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
16-11-1992	07. 12. 92
International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	Dagmar Frank Mme Dagmar FRANK

International Application No PCT/US 92/06681

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	Gann, The Japanese Journal of Cancer Research, vol. 64, no. 4, August 1973, F. KANZAWA et al.: "Antitumor activity of haloacetylcarbazole derivatives", pages 391-396, see pages 392-393, tables I+II; page 394 ---	1-2,7,9 ,19,23
X	GB,A, 850334 (CHAS. PFIZER & CO. INC.) 5 October 1960, see pages 1-3; claims 10-12,14	19-21
Y	---	5-9
X	BE,A, 569697 (S.A. RECHERCHE ET INDUSTRIE THERAPEUTIQUES) 24 January 1959, see pages 4,8; claims 9,10,18,22,23	19-21
Y	---	5-9
T	Journal of Medicinal Chemistry, vol. 35, no. 18, 4 September 1992, American Chemical Society, K.N. THIMMAIAH et al.: "Synthesis and chemical characterization of N-substituted phenoxazines directed toward reversing vinca alkaloid resistance in multidrug-resistant cancer cells", pages 3358-3364, see whole article -----	1-23

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9206681

SA 63482

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 27/11/92
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB-A- 850334		None	
BE-A- 569697		None	

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